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DNA damage of lymphocytes in experimental chronic renal failure: Beneficial effects of losartan

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Background. Kidney diseases are associated with the accumulation of various uremic toxins increasing the oxygen free radical (OFR) activity with a number of serious consequences. One of them could be the impairment of DNA stability with the increased formation of DNA breaks.

Methods. The study was performed in 4/6 kidney ablation rat nephropathy lasting for three months. The results of sham-operated (Sham), remnant kidney (Nx), and Nx treated by losartan (NxL) were compared.

Results. Nx significantly increased blood pressure, plasma creatinine, urea, hippurate, malondialdehyde (MDA), lipofuscin (LF), and the number of DNA breaks of lymphocytes. Losartan decreased the rise of blood pressure and inhibited the rise of creatinine plasma concentration but not of other variables, while it markedly inhibited the number of DNA breaks (Sham 15.9 ± 1.1, Nx 54.5 ± 1.7, $P < 0.001$; Nx/Sham, NxL 23.3 ± 2.6 $P < 0.001$, NxL/Sham and $P < 0.001$ NxL/Nx).

Conclusions. The 4/6 kidney ablation nephropathy increases the susceptibility of lymphocyte DNA to breaks, and losartan inhibits the number of breaks by a mechanism independent on glomerular filtration, accumulation of MDA or LF (markers of oxidative stress), and hippurate (marker of the accumulation of middle molecular substances). An independent mechanism, probably the interference with proliferation, is suggested.

Chronic renal failure (CRF) is associated with the retention of various uremic toxins, increasing the oxygen free radical (OFR) activity, diminishing antioxidant capacity, reducing OFR-inactivating enzyme activity [1], and the accumulation of advanced glycated end products (AGEs) [2]. Changes in circulating levels of catabolic cytokines and profibrotic growth factors like insulin-like growth factor-1 (IGF-1), interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), fibroblast growth factor, and platelet-derived growth factor (PDGF) could also participate in the increased OFR activity [3]. Moreover, recently, a radical new idea about the possibility of direct impairment of DNA by the increased OFR activity, supported by the studies on bacteria and mammalian cells, has been postulated [4]. Independently, an impairment of DNA repair has been found [5]. All of these factors could contribute to DNA damage with serious consequences for kidney disease progression.

The progression of kidney diseases could be accelerated also by diverse factors, including systemic and glomerular hypertension, proteinuria, and stimulation of renin-angiotensin-aldosterone system (RAS). RAS blockade either by angiotensin-converting enzyme inhibitors or by angiotensin II receptor type 1 (AT_1 receptor) blockade has a great importance in renal protection as well as in cardiac and vascular remodeling [6, 7]. Thus, a pilot study focused on DNA damage in rat remnant kidney model was performed, and the effect of AT_1 receptor blocker losartan was evaluated.

METHODS

Animals

Thirty-three male Wistar rats (180 to 220 g; Velaz, Prague, Czech Republic) underwent 4/6 kidney ablation [8] and were randomized into a losartan-treated group (NxL, $N = 16$, 20 mg/L in drinking water; Merck Sharp & Dohme, Rahwaym NJ, USA) compared with a placebo group (Nx, $N = 17$). Sham-operated rats served as controls (Sham, $N = 16$). Pair feeding was performed. The duration of the study was three months.

Investigations

Systolic blood pressure (SBP) was measured by tail plethysmography under light ether anesthesia prior to operation and sacrifice. Blood collected from abdominal aorta under thiopental narcosis before sacrifice was ana-

Key words: DNA break, ablation nephropathy, uremic toxin, kidney ablation, lymphocyte DNA, oxidative stress.

Table 1. Pertinent data in losartan-treated remnant kidney rats

	Sham	Nx	NxL
Blood pressure *mm Hg*	112 ± 2.3	120 ± 5.4[a]	103 ± 16.5[a]
Serum creatinine *μmol/L*	46.0 ± 1.9	102 ± 14.2[a]	88.2 ± 6.2[a]
Urea *mmol/L*	6.1 ± 0.2	13.34 ± 2.5[b]	18.18 ± 6.6[c]
Proteinuria *g/L*	10 ± 1	20 ± 3[a]	20 ± 4[a]
Albumin *g/L*	26.6 ± 0.8	22.6 ± 1.1[a]	22.5 ± 0.8[a]
Hippurate *μmol/L*	8.1 ± 0.5	15.1 ± 2.7[a]	16.4 ± 1.7
Malondialdehyde *μmol/L*	2.2 ± 0.1	5.4 ± 1.0[a]	6.2 ± 1.3[b]
Lipofuscin *AU*	7.9 ± 0.2	9.2 ± 1.3	9.4 ± 0.6
DNA breaks *%DNA in tail*	14.72 ± 1.22	54.53 ± 2.11[c]	23.29 ± 3.28[c]

[a] $P < 0.05$ vs. Nx
[b] $P < 0.01$ vs. Sham
[c] $P < 0.001$ vs. Nx

lyzed for serum creatinine and urea concentrations (Vitros 250; Johnson & Johnson, Rochester, NY, USA). The malondialdehyde (MDA) concentration was determined by the high-performance liquid chromatography (HPLC) method with fluorometric detection [9]. Lipofuscin (LF) was determined fluorometrically [10]. Hippurate was determined by the HPLC method with ultraviolet detection [11].

Comet assay

The comet assay was used to detect the DNA breaks. Lymphocytes isolated by centrifugation on a Ficoll-based density gradient were embedded in agarose on a microscope slide and lyzed in a solution containing Triton X-100 and 2.5 mmol/L NaCl. The resulting nucleoides were fractioned by electrophoresis under alkaline conditions. The forming comets were stained with 4,6 diamino-2-phenylindole (DAPI) and were viewed by fluorescence microscopy. One hundred comets in each gel were analyzed visually. The overall score for each slide was between 0 (undamaged) and 4 (maximally damaged). Although these units are arbitrary, they were related to the tail intensity, which itself is a function of break frequency [1, 2, 5, 8].

Statistics

Results are expressed as mean values ± SEM. Differences between the groups were evaluated by analysis of variance (ANOVA) followed by the least significant difference test. $P < 0.05$ was considered significant.

RESULTS

The administration of losartan (AT_1 receptor antagonist) to remnant kidney rats resulted in a significant improvement of systolic blood pressure (SBP) and renal function (Table 1). SBP in the NxL group was significantly lower compared not only with the Nx group, but even with the Sham group. Renal function was impaired in both remnant kidney groups, although in case of serum creatinine, losartan only partially prevented it. Serum urea concentration was elevated in both Nx and NxL groups. Both MDA and LF concentrations were increased in Nx, and losartan did not influence their concentrations.

DNA damage (measured by DNA breaks) was very strong. Surprisingly, the treatment with losartan almost fully prevented any damage (Table 1).

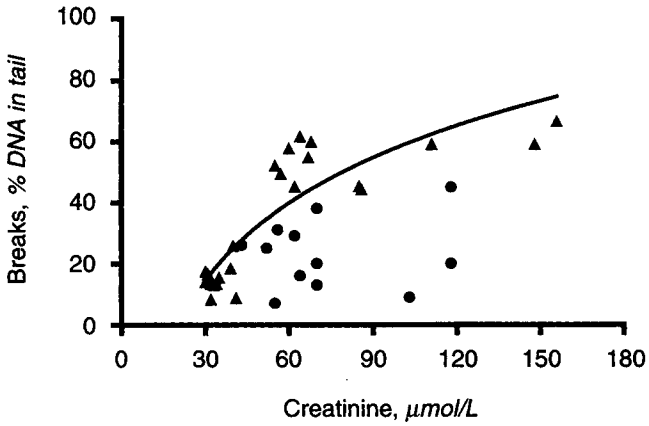

Fig. 1. Relationship between DNA breaks and plasma creatinine concentration. Symbols are: (▲) Sham operated (Sham) + 4/5 nephrectomized (Nx) rats; (●) nephrectomized taking losartan (NxL) rats. $r = 0.8824$; $P < 0.001$.

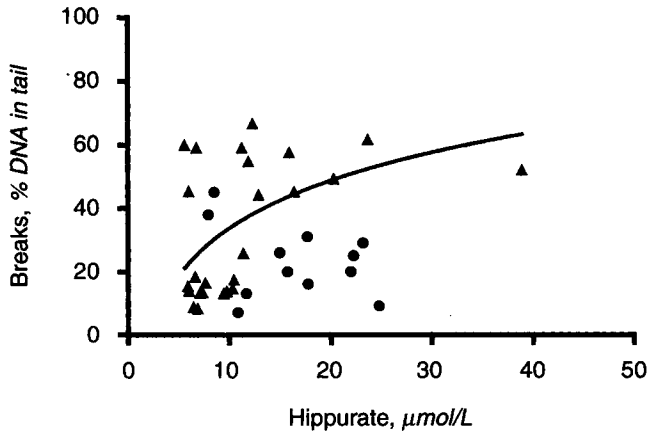

Fig. 2. Relationship between DNA breaks and plasma hippurate concentration. Symbols are: (▲) Sham + Nx rats; (●) NxL rats. $r = 0.5052$; $P < 0.01$.

Relationships between DNA breaks and markers of renal failure

A highly significant relationship was found between the DNA breaks and serum creatinine concentration in Sham + Nx groups, but losartan decreased the number of breaks getting the values outside of the relationship (Fig. 1). A similar independence was found also between the DNA breaks and urea (data not presented), plasma hippurate (Fig. 2), and MDA (Fig. 3).

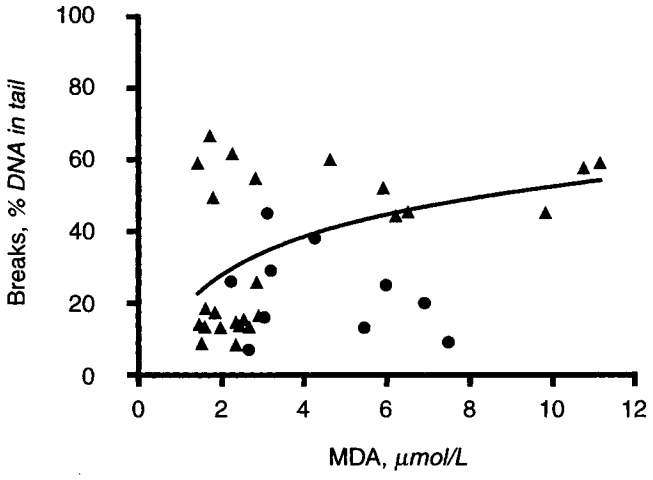

Fig. 3. Relationship between DNA breaks and plasma malondialdehyde (MDA) concentration. Symbols are: (▲) Sham + Nx rats; (●) NxL rats. $r = 0.4723$; $P < 0.05$.

DISCUSSION

Consequences of reduced renal mass

Chronic renal failure, besides reducing kidney function, accelerates the production of OFRs, increases plasma lipid peroxidation product MDA, decreases total antioxidant capacity, and impairs the antioxidant enzyme system [1, 12]. The presence of high levels of oxidized proteins (AOPPs) in the plasma of hemodialyzed patients, highly correlating with AGEs and dityrosine, was described [13]. Another important feature associated with uremia is the accumulation of AGEs, including carboxymethyl-lysine (CML) and pentosidine. Since CML and pentosidine formation are closely linked to oxidative processes, their production is accelerated under oxidative stress even in the absence of glucose [14]. AGEs accumulation in glomeruli is associated with increased synthesis of extracellular matrix by mesangial cells [15], increased nuclear factor binding of the promoter of the collagen IV gene, and a stimulated synthesis of smooth muscle actin [5]. AGEs also directly bind to DNA, increase mutations [16], and impair DNA repair [5]. Recently, the possibility of direct genetic response to oxidative stress has been stressed [4] and turned our attention to the possibility of decreased resistance of DNA to breaks in renal failure. It was found that the number of DNA breaks in lymphocytes isolated from animals after 4/6 kidney ablation increased after the oxidation stress and correlated with plasma creatinine, hippurate, and MDA concentrations.

Effect of losartan

The protective effect of losartan on DNA breaks of such a degree is surprising, although some form of protection has been expected. It decreases serum creatinine concentration, that is, it improves glomerular filtration rate, but the whole losartan group is outside of the regression line, calculated on the values of Sham + Nx group. The similar difference also is apparent in the case of hippurate, which is a good marker of the accumulation of middle molecules [17], and MDA, which is a marker of oxidative stress. A possible cause could have been the decreased blood pressure in losartan group. However, hypertension in Nx rats was mild, and the administration of losartan did not reflect in the improvement of any of the determined markers, with the exception of plasma creatinine. Moreover, no relationship between the blood pressure and DNA breaks was found (data not presented). Thus, an additional effect of losartan, that is, antiproliferative effect, not measured in this study, could be suggested. This result is of outstanding importance because of inhibition of DNA repair in renal failure [5]. In any case, the increased risk of DNA breaks opens a new area of research that could be of remarkable value for kidney disease research.

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APPENDIX

Abbreviations used in this article are: AGEs, advanced glycation end products; AT_1, angiotensin II type 1 receptor; CML, carboxymethyl-lysine; CRF, chronic renal failure; HPLC, high pressure liquid chromatography; LF, lipofuscin; MDA, malondialdehyde; Nx, remnant kidney; NxL, remnant kidney treated with losartan; OFR, oxygen free radical; RAS, renin-angiotensin system; SBP, systolic blood pressure.

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